13544

034650

Chemical:	**1-Octadecanaminium, N,N-dimethyl-N-(3-(t**
PC Code:	**107401**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/13/93**
File ID:	**TX010556**
Accession Number:	**412-02-0281**

HED Records Reference Center
04/23/2002

13000

892B

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

010556

SEP 13 93

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: DC 5700 Hydrolysate: review of three mutagenicity studies and one range finding study.

HED Project No.: 0-0289
Tox.Chem No.: 892B
MRID No.: 412968-01; 02; 03; 04
DP Barcode: D149040
Submission No.: S283375
PC Code: 107401

From: John C. Redden, Toxicologist 9/1/93
Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

To: James Wilson, PM 31
Disinfectants Branch
Registration Division (H7505C)

Thru: Karen L. Hamernik, Ph.D. 9/1/93
Section Head Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

KB 9/3/93

CONCLUSIONS:

In MRID 412968-01, "Mutagenicity Test on DC 5700 TK +/- Mouse Lymphoma Forward Mutation Assay," DC 5700 was evaluated in two nonactivated assays with nine doses ranging from 0.5 to 13.0 µg/ml, and in two S9-activated assays. Doses in the first S9-activated assay ranged from 0.5 to 60.0 µg/ml and doses in the second S9- activated assay ranged from 0.5 to 30.0 µg/ml. The reviewer reports that no definitive conclusions can be reached due to, "...the inconsistency of the data and the conflicting results." No information was provided as to compound stability. Since the findings are equivocal, the study does not satisfy the requirements for Gene Mutations (84-2). The study is graded **unacceptable**. The study author reports that a repeat assay is in progress.

In MRID 412968-02, "Single Acute Exposure Dose Selection Study on DC 5700 Hydrolysate, Reference# E6653-8," the study objective was to determine doses for an in vivo micronucleus assay (MRID No.

412968-03). The study authors concluded that the results from this range finding study were sufficient to select doses for the mouse bone marrow micronucleus assay (see MRID No. 412968-03). The test material was administered by gavage to ICR mice 3/sex/group at doses of 500, 1625, 2750, 3875, and 5000 mg/kg body weight. One female died in the 3875 mg/kg dose group. The other animals appeared to be healthy until termination of the three day study. However, the Agency does not regulate on range finding studies and can only acknowledge the receipt of the data.

In MRID 412968-03, "Mutagenicity Test on DC 5700 Hydrolysate Ref. #E6653-8 in vivo Mouse Micronucleus Assay," groups of five male and five female mice were administered single doses of 500, 2500, or 5000 mg/kg DC 5700 hydrolysate. No compound related toxicity was observed in either sex at the high dose, and there were no significant increases in the percentage of micronucleated polychromatic erythrocytes in the bone marrow cells harvested 24, 48, and 72 hours following exposure to the three doses of the test material. Similarly, there was no evidence of compound cytotoxicity in the target cells. As there was no overt toxicity observed in the animals nor cytotoxicity in the target cells, DC 5700 hydrolysate was assayed to an adequately high dose, but failed to induce a clastogenic effect. The study is classified as **acceptable** for structural chromosome aberrations (84-2).

In MRID 412968-04, "Mutagenicity Test on Dow Corning 5700 Hydrolysate in Rat Hepatocyte Unscheduled DNA Synthesis Assay," two independent assays were conducted using DC 5700 hydrolysate. The dose range in the first assay was 0.025 to 1.02 µg/ml, and the dose range in the second assay was 0.063 to 2.01 µg/ml. It is reported that neither assay induced a significant increase in the net nuclear grain counts of treated rat hepatocytes. Both assays had significant flaws as the reviewer points out:

> "However, the first assay was considered invalid because of the high frequency of net nuclear grains in the solvent control group. The second assay was also compromised by the lack of a definitive cytotoxic effect at the highest assayed level (2.01 µg/ml). Although a slight reduction in cell survival (23.3%) was seen at this level, we question the evidence for cytotoxicity because this finding conflicts with the study author's statement that severe cytotoxicity, which prevented the scoring of hepatocytes, occurred at doses >1.02 µg/ml in the first assay."

Additional study deficiencies were:

> 1) Failure to provide data to support the conclusion that doses > 1.02 µg/ml were "excessively" cytotoxic;
>
> 2) Actual grain count and Standard Deviation was not presented; and

3) Information on test material stability, storage conditions, or analytical data to demonstrate actual concentrations was not provided.

This study does not satisfy guideline requirements for 84-2 other genotoxic effects. The study is classified as **unacceptable**, and it is suggested that the study be repeated. Clear evidence of cytotoxicity should be demonstrated.

EPA No.: 68D80056
DYNAMAC No.: 287-C
TASK No.: 2-87C
August 17, 1990

DATA EVALUATION RECORD

DOW CORNING® 5700

Mutagenicity--Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L. McLellan for

Date: 8-17-90

EPA No.: 68D80056
DYNAMAC No.: 287-C
TASK No.: 2-87C
August 17, 1990

DATA EVALUATION RECORD

DOW CORNING® 5700

Mutagenicity--Unscheduled DNA Synthesis Assay
in Primary Rat Hepatocytes

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 8-17-90

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan for
Date: Aug. 17, 1990

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: Aug. 17, 1990

Irving Mauer
~~Henry Spencer,~~ Ph.D.
EPA Reviewer, Section
Toxicology Branch I
(H-7509C)

Signature:
Date: 08-24-93

KAREN BRANCHOK, Ph.D.
~~Marion Copley, D.V.M.~~,
~~D.A.B.T.~~
EPA Section Head, Section 2
Toxicology Branch I
(H-7509C)

Signature:
Date: 8/24/93

DATA EVALUATION RECORD

CHEMICAL: Dow Corning® 5700.

STUDY TYPE: Mutagenicity--unscheduled DNA synthesis assay in primary rat hepatocytes.

MRID NUMBER: 412968-04.

TEST MATERIAL: Dow Corning® 5700 Hydrolysate.

SYNONYM: 3-(Hydroxysilyl)propyl octadecyldimethyl ammonium chloride.

STUDY NUMBER: 10864-0-447.

SPONSOR: Dow Corning Corp., Midland, MI.

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD.

TITLE OF REPORT: Mutagenicity Test on Dow Corning® 5700 Hydrolysate in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay.

AUTHOR: M. A. Cifone.

REPORT ISSUED: October 24, 1989.

CONCLUSIONS - Executive Summary:

Under the conditions of two independent assays, six doses of Dow Corning® 5700 ranging from 0.025 to 1.02 μg/mL in the first assay and six doses ranging from 0.063 to 2.01 μg/mL in the repeat assay did not induce an appreciable increase in the net nuclear grain counts of treated rat hepatocytes. However, the first assay was considered invalid because of the high frequency of net nuclear grains in the solvent control group. The second assay was also compromised by the lack of a definitive cytotoxic effect at the highest assayed level (2.01 μg/mL). Although a slight reduction in cell survival (23.3%) was seen at this level, we question the evidence for cytotoxicity because this finding conflicts with the study author's statement that severe cytotoxicity, which prevented the scoring of hepatocytes, occurred at doses >1.02 μg/mL in the first assay.

In addition, the study was compromised for the following reasons:

1. Data to support the conclusion that doses >1.02 μg/mL were "excessively" cytotoxic were not reported.

2. Presentation of average values without the actual grain counts or some indication of variability (standard deviations) is not an acceptable practice.

3. No information on test material stability, storage conditions, or analytical data to support actual concentrations used in the study were provided.

We assess, therefore, that the study does not fully satisfy guideline requirements for genetic effects, Category III, Other Mutagenic Mechanisms.

Study Classification: The study is unacceptable. We recommend that the assay be repeated and that the high dose used in the repeat study be selected to show clear evidence of a cytotoxic effect.

A. MATERIALS:

1. Test Material:

Name:	Dow Corning® 5700 Hydrolysate
Description:	White powder
Batch No.:	BN029263-642394
Purity:	81.08%
Contaminants:	See Appendix A, Test Material Composition Information
Solvent used:	Ethanol (ETOH)
Other comments:	The report indicated that the test material was insoluble in water, insoluble in

dimethylsulfoxide at ≈25 mg/mL but soluble at this concentration in ETOH. The solvent selected for the assay was, therefore, ETOH. Solutions of the test material were prepared fresh on the day of use. Neither storage conditions nor test material stability information was reported.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the livers of adult male Fisher 344 rats purchased either from Harlan Sprague-Dawley, Inc. (Trial 1; 231.0 g) or from Charles River Breeding Laboratories, Inc. (Trial 2; 263.7 g).

3. Positive Control: 2-Acetylaminofluorene (2-AAF) at 0.10 µg/mL was used as the positive control.

B. STUDY DESIGN:

1. Cell Preparation:

a. Perfusion Technique: The livers were perfused with Hanks' balanced salts containing 0.5 mM EGTA and HEPES buffer, pH 7.2, for 4 minutes and with incomplete William Medium E (WMEI) containing 50 to 100 U/mL collagenase for 10 minutes. Livers were excised, removed to a culture dish containing WMEI and collagenase, and mechanically dispersed to release the hepatocytes.

b. Hepatocyte Harvest/Culture Preparation: Recovered cells were centrifuged, resuspended in WME containing 10% fetal calf serum and antibiotics (WME+), counted, and aliquoted (0.5×10^6 cells/3 mL WME+) onto plastic coverslips. The cultures were placed in a humidified, 37°C, 5% CO_2 incubator for a 1.8- to 2.0-hour attachment period. Unattached cells were removed; viable cells were refed and established as monolayer cultures.

2. Dose Selection: Initially, 15 concentrations of the test material were assayed. When the viability estimate was obtained (20.4 hours after treatment initiation), at least six of these doses were chosen for analysis of nuclear labeling, starting with the highest dose that resulted in a sufficient number of survivors with intact morphologies and proceeding to successively lower doses.

3. UDS Assay:

a. Treatment: Five replicate monolayer cultures were exposed to the selected doses of the test material, negative (ETOH), or positive controls (2-AAF, 0.10 µg/mL) for 18.4 or 19.2 hours in WMEI containing 5 µCi/mL [^{3}H]thymidine. Treated monolayers were washed twice with WMEI; two of the five replicates for each treatment group were used to determine cytotoxicity. These cultures were refed, reincubated, and monitored for cytotoxicity at 20.4 or 21.8 hours posttreatment by trypan blue exclusion.

b. UDS Slide Preparation: The remaining cultures were washed with media containing 1 mM thymidine. Treated hepatocytes, attached to coverslips, were exposed to 1% sodium citrate for 10 minutes, fixed in acetic acid:ethanol (1:3), dried, and mounted.

c. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak NTB2 emulsion, dried for 7 to 10 days at 4°C in light-tight desiccated boxes, developed in Kodak D-19, fixed, stained with Williams' modified hematoxylin and eosin, coded, and counted.

d. Grain Counting: The nuclear grains of 150 morphologically normal cells for each test dose and negative and positive controls were counted microscopically. Net nuclear grain counts were determined by subtracting the nuclear grain counts of each cell from the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus.

4. Evaluation Criteria:

a. Assay Validity: For the assay to be considered valid, the following criteria must be satisfied: (1) hepatocytes recovered from the perfusion step and monolayer cultures used for the assay must show ≥70% viability; (2) the negative control should have net nuclear grain counts of -5.0 to 1.0, and ≤10% of the cells should contain ≥6 grains/nucleus; (3) the positive control must demonstrate the sensitivity of the test system to detect UDS; (4) data must be obtained from at least two replicate cultures/dose; and (5) the highest dose must show cytotoxicity, the limit

of solubility, or reach the maximum recommended dose for this assay (5 mg/mL).

b. Positive Response: The assay was considered positive if (1) the increase in the average net nuclear grain count was ≥6 grains/nucleus over the negative control value, and (2) the percent of nuclei with ≥6 grains exceeded 10 percent of the negative control population.

C. REPORTED RESULTS:

UDS Assay: Two trials were conducted. For the initial test, doses ranging from 0.005 to 254 μg/mL were assayed. The report indicated that doses >50.8 μg/mL were insoluble in culture medium. The report further indicated that exposure to doses >1.02 μg/mL resulted in severe cytotoxicity; however, no data were presented to support this statement.

For the remaining doses, survival ranged from 72.5% at 1.02 μg/mL to 100.0% at doses ≤0.025 μg/mL. Based on these findings, the six doses selected for the evaluation of nuclear labeling were 0.025, 0.058, 0.102, 0.254, 0.508, and 1.02 μg/mL. Results presented in Table 1 indicate that cells exposed to these levels did not show an increase in net nuclear grains compared to the solvent control group. However, our reviewers noted that the background solvent control frequency of net nuclear grains was high (0.42 net nuclear grains) and beyond the historical range for the reporting laboratory (-2.48 to 0.38 net nuclear grains). Similarly, the presence of cells with ≥6 grains was elevated in the control group (5.3%) but within the historical range (0-9.3%). A slight increase in the percent of cells with ≥6 grains was also seen in the highest dose group (1.02 μg/mL) scored for UDS. We assume, therefore, that the repeat assay was performed because of the high background frequency in the solvent control group.

For the repeat test, 10 doses ranging from 0.004 to 2.01 μg/mL were assayed. Survival was dose dependent and ranged from 76.7% at 2.01 μg/mL to ≥96.2% at doses ≤0.063 μg/mL. The percent survival for the 2.01-μg/mL dose group (76.7%) conflicted with the earlier comments of the study author, indicating that doses in excess of 1.02 μg/mL were severely cytotoxic. Since neither the test material dilution scheme nor cytotoxic assessment was presented for doses higher than 1.02 μg/mL in the first trial, we were unable to determine the extent to which the results from the first and second trials

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assays With Dow Corning® 5700 Hydrolysate

Treatment	Dose (µg/mL)	Cells Scored	Percent Survival (20.4 or 21.8 hours post-exposure)	Average Nuclear Grain Count	Average Cytoplasmic Grain Count	Average Net Nuclear Grain Count[a]	Average Percent Nuclei w/ ≥6 Grains
Solvent control							
Ethanol	--	150[b]	100	10.09	9.67	0.42	5.3
	--	150[c]	100	10.60	10.91	-0.31	2.7
Positive control							
2-Acetylaminofluorene	0.1	150[b]	91.2	32.97	7.98	24.99[d]	98.7
	0.1	150[c]	77.7	32.88	12.11	20.77[d]	98.0
Test Material							
Dow Corning® 5700 Hydrolysate	1.02[e,f]	150[b]	72.5	11.42	11.65	-0.23	6.0
	1.01[e]	150[c]	81.9	11.52	12.44	-0.92	0.0
	2.01	150[c]	76.7	14.38	14.09	0.29	8.0

[a]Average net nuclear grain counts = Average nuclear grain count - Average cytoplasmic grain count.

[b]Results from the initial assay.

[c]Results from the repeat assay.

[d]Fulfills reporting laboratory's criteria for a positive effect.

[e]Results for lower levels (0.025, 0.058, 0.102, 0.254, and 0.508 µg/mL in the initial assay and 0.063, 0.126, 0.251, and 0.502 µg/mL in the repeat assay) did not indicate a genotoxic effect.

[f]Higher concentrations used in the initial assay (actual doses not reported but assayed up to 254 µg/mL) were reported to be severely cytotoxic and, therefore, were not scored.

varied. As shown in Table 1, a slight increase in both net nuclear grains and the percentage of cells with ≥6 grains/nucleus was observed in cultures exposed to 2.01 μg/mL of the test material. However, the increases did not meet the minimum criteria for a positive response (i.e., ≥6 net nuclear grain count and ≈10% of the cells have ≥6 net nuclear grains). Based on the overall results the study author concluded:

> "The test material, Dow Corning 5700 Hydrolysate, did not induce changes in the nuclear labeling of rat primary hepatocytes in two independent trials for an applied concentration range of 2.01 μg/mL to 0.025 μg/mL. Dow Corning® 5700 Hydrolysate was therefore evaluated as inactive in the Rat Primary Hepatocyte UDS Assay.

D. REVIEWERS' DISCUSSION/INTERPRETATION OF STUDY RESULTS:

We assess that the results of the initial assay are not valid because of the high background UDS frequency in the solvent control group. The findings for the second assay did not suggest that Dow Corning® 5700 was genotoxic; however, there are concerns regarding the selection of the high dose. Although a slight reduction in cell survival (23.3%) was observed at the highest dose (2.01 μg/mL) used in the repeat trial, this finding conflicts with the statement made by the study author that "the test material was excessively cytotoxic at test concentrations exceeding 1.02 μg/mL."

Since compound stability was not reported and all data from the cytotoxicity assessment were not included, the cytotoxic level can not be resolved. Furthermore, analytical verification of the actual doses used in the study were not reported; therefore, there is no supporting evidence of dose equivalency between the two trials.

We conclude, therefore, that the lack of a genotoxic effect in the absence of a definitive cytotoxic response provides no assurance that the test material has been adequately evaluated.

The acceptability of the study was further compromised because of the method used to report UDS assay results. Presentation of average values without some indication of variability (standard deviations) is not an acceptable practice.

Based on the above considerations, it was concluded that the assay is unacceptable.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated October 24, 1989.

F. CBI APPENDIX: Appendix A, Test Material Composition Information, CBI p. 28; Appendix B, Materials and Methods, CBI pp. 12-18.

Page _____ is not included in this copy.

Pages 15 through 24 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

___ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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EPA No.: 68D80056
DYNAMAC No.: 287-B
TASK No.: 2-87B
April 20, 1990

DATA EVALUATION RECORD

DOW CORNING 5700

Mutagenicity--Mouse Micronucleus Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L. McLellan for

Date: April 20, 1990

EPA No.: 68D80056
DYNAMAC No.: 287-B
TASK No.: 2-87B
April 20, 1990

DATA EVALUATION RECORD

DOW CORNING 5700

Mutagenicity--Mouse Micronucleus Assay

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-19-90

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan for
Date: April 20, 1990

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: April 20, 1990

Henry Spencer, Ph.D.
EPA Reviewer, Section II
Toxicology Branch I
(H-7509C)

Signature: Henry Spencer
Date: April 23, 1990

~~Marion Copley, D.V.M., D.A.B.T.~~ KAREN HAMILTON, PhD
EPA Section Head, Section ~~II~~ 3
Toxicology Branch I
(H-7509C)

Signature: K L A
Date: 8/30/93

DATA EVALUATION RECORD

CHEMICAL: Dow Corning 5700.

STUDY TYPE: Mutagenicity--Mouse micronucleus assay.

MRID NUMBER: 412968-03C.

TEST MATERIAL: DC 5700 hydrolysate.

SYNONYM: 3-(Hydroxysilyl)propyl octadecyldimethyl ammonium chloride.

STUDY NUMBER: 10864-0-455.

SPONSOR: Dow Corning Corp., Midland, MI.

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD.

TITLE OF REPORT: Mutagenicity Test on DC 5700 Hydrolysate Ref. #E6653-8 *in vivo* Mouse Micronucleus Assay.

AUTHOR: Ivett, J. L.

REPORT ISSUED: September 15, 1989.

CONCLUSIONS - Executive Summary:

Groups of five male and five female mice were administered single oral gavage doses of 500, 2500, or 5000 mg/kg DC 5700 hydrolysate. No evidence of compound-related toxicity was observed in the high-dose animals of either sex. There were no significant increases in the percentage of micronucleated polychromatic erythrocytes in the bone marrow cells harvested 24, 48, and 72 hours following exposure to the three doses of the test material. Similarly, there was no evidence of compound cytotoxicity in the target cells. We conclude, therefore, that in the absence of overt toxicity in the animals and cytotoxicity in the target cells, DC 5700 hydrolysate was assayed to an adequately high dose but failed to induce a clastogenic effect.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material:

Name:	DC 5700 Hydrolysate Ref. No. E6653-8
Description:	White powder
Lot #:	BN029263-642394
Purity:	81.08%
Contaminants:	See Appendix A, Test Material Composition Information
Solvent used:	Corn oil
Other comments:	The report indicated that the solubility of the test material was determined prior to the performance of a dose range-finding assay. No information on test material stability or storage conditions was reported.

2. Control Materials:

Negative/Route of Administration: None.

Vehicle/Final Concentration/Route of Administration: Corn oil was administered once by oral gavage at a dosing volume of 10 mL/kg.

Positive/Final Concentration/Route of Administration: Cyclophosphamide (CP) at 80 mg/kg was administered once via oral gavage.

3. Test Compound: Route of administration: oral gavage.

Dose levels used: 500, 2500, and 5000 mg/kg.

Note: The three doses for the micronucleus assay were based on the results of a preliminary dose range-finding study; no further information was provided.

4. Test Animals:

a. Species: Mouse
Strain: ICR
Mean weight: 27.4-39.2 g (males), 23.7-29.6 g (females)
Age: ≈8.5 weeks
Source: Harlan Sprague-Dawley, Inc., Frederick, MD.

b. No. animals used per dose/group: five males, five females. An additional secondary group of five male and five female mice were administered the high test material dose (5000 mg/kg) and were to be used only in the event that high-dose animals in the primary group died.

c. Properly maintained: YES.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound
Dosing: __x__ once _____ twice (24 hr apart)

Sampling (after last dose):
__x__ 24 hr __x__ 48 hr __x__ 72 hr

b. Vehicle control
Dosing: __x__ once _____ twice (24 hr apart)

Sampling (after last dose): __x__ 24 hr ____ 48 hr _____ 72 hr

c. Positive control
Dosing: __x__ once _____ twice (24 hr apart)

Sampling (after last dose): __x__ 24 hr ____ 48 hr _____ 72 hr

2. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):
No. of polychromatic erythrocytes (PCE) examined per animal: 1000.
No. of normochromatic erythrocytes (NCE; more mature RBCs): The number of NCEs was determined per 1000 PCEs per animal.

C. REPORTED RESULTS:

1. Preliminary Dose Range-Finding Study: The report indicated that a preliminary dose range-finding study was conducted, and based on the results, the three doses selected for the micronucleus assay were 500, 2500, and 5000 mg/kg of the test material. No further information was furnished.

2. Micronucleus Assay: Two trials of the micronucleus assay were performed. The first study was aborted because of technical difficulties with slide preparation; therefore, the assay was repeated. In the repeat assay, one male in the mid-dose (2500 mg/kg) group died within 22 hours of dosing. Also, in the mid-dose group, one male showed signs of prostration and labored breathing within 18 hours of treatment; the animal remained weak with signs of a yellow discharge around the eyes until the scheduled sacrifice. An additional mid-dose male developed a distended abdomen within 41 hours of dosing, which persisted until sacrifice. No signs of compound-related effects were, however, noted in the high or low test material dose groups; it appeared that the mortality and other observed symptoms in the mid-dose groups were not compound related.

   Representative results from the repeat micronucleus assay are presented in Table 1. As shown, no significant increases in the percentage of micronucleated polychromatic erythrocytes (MPEs) were observed in male or female mice administered a single oral gavage dose of 5000 mg/kg of the test material and sacrificed 24, 48, and 72 hours posttreatment. The ratio of PCE:NCE for high-dose group animals did not indicate that the test material adversely affected erythropoiesis. Similar results were obtained for the low- (500 mg/kg) and mid- (2500 mg/kg) dose groups. By contrast to the uniformly negative test material results, males and females exposed to the positive control (80 mg/kg CP) showed significant ($p < 0.05$) increases in the percentage of MPEs. From these results, the study author concluded:"The test material, DC 5700 Hydrolysate Ref. No. E6653-8, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse bone marrow micronucleus test."

TABLE 1. Representative Results of the Micronucleus Assay in Mice with DC 5700 Hydrolysate

Substance	Dose	Exposure Time[a] (hours)	No. of Animals Analyzed per Group[b]	No. of PCEs[b] Analyzed per Group	Total No. of MPEs[b] per Group	Mean Percent MPEs[b] ± S.E.	Mean PCE:NCE[b] ± S.E.
Vehicle Control							
Corn oil	10 mL/kg	24	5M	5000	5	0.10 ± 0.06	0.57 ± 0.13
			5F	5000	10	0.20 ± 0.08	0.70 ± 0.04
Positive Control							
Cyclophosphamide	80 mg/kg	24	5M	5000	44	0.88 ± 0.27*	0.70 ± 0.15
			5F	5000	65	1.30 ± 0.34*	0.48 ± 0.05
Test Material							
DC 5700 Hydrolysate	5000 mg/kg[c]	24	5M	5000	6	0.12 ± 0.06	0.67 ± 0.11
			5F	5000	6	0.12 ± 0.08	0.62 ± 0.05
		48	5M	5000	7	0.14 ± 0.04	0.25 ± 0.07
			5F	5000	4	0.08 ± 0.05	0.99 ± 0.31
		72	5M	5000	2	0.04 ± 0.02	0.65 ± 0.07
			5F	5000	1	0.02 ± 0.02	0.65 ± 0.10

[a]Time after compound administration.

[b]Abbreviations used:
PCE--Polychromatic erythrocytes
MPE--Micronucleated polychromatic erythrocytes
NCE--Normochromatic erythrocytes

[c]Similar results were obtained in the low- (500 mg/kg) and mid- (2500 mg/kg) dose groups; therefore, the data from the high- (5000 mg/kg) dose group were selected as representative.

*Significantly higher than the negative control ($p < 0.05$) by analysis of variance.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the test material was assayed over an appropriate concentration range with no evidence of a clastogenic effect in a well-controlled study. The sensitivity of the test system to detect increased frequencies of PCEs with micronuclei was adequately demonstrated by the significant findings in males and females administered the positive control, 80 mg/kg CP. We conclude, therefore, that the study author's interpretation of the data was correct, and that DC 5700 hydrolysate is not clastogenic in this test system.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated September 15, 1989.

F. CBI APPENDIX: Appendix A, Test Material Composition Information, CBI p. 26; Appendix B, Materials and Methods, CBI pp. 12-16.

Page _____ is not included in this copy.

Pages __33__ through __40__ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

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EPA No.: 68D80056
DYNAMAC No.: 287-A1/A2
TASK No.: 2-87A1/A2
August 17, 1990

DATA EVALUATION RECORD

DOW CORNING® 5700

Mutagenicity--Mouse Lymphoma Forward Mutation Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L. McLellan for

Date: Aug 18 1992

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DATA EVALUATION RECORD

DOW CORNING® 5700

Mutagenicity--Mouse Lymphoma Forward Mutation Assay

REVIEWED BY:

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Dynamac Corporation

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Signature:
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DATA EVALUATION RECORD

CHEMICAL: Dow Corning® 5700.

STUDY TYPE: Mutagenicity--Mouse lymphoma forward mutation assay.

MRID NUMBER: 412968-01C.

TEST MATERIAL: DC® 5700 Hydrolysate.

SYNONYM: 3-(Hydroxysilyl)propyl octadecyldimethyl ammonium chloride.

STUDY NUMBER: 10864-0-431.

SPONSOR: Dow Corning Corp., Midland, MI.

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD.

TITLE OF REPORT: Mutagenicity Test on DC 5700 Hydrolysate in the L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay.

AUTHOR: R. R. Young.

REPORT ISSUED: October 25, 1989.

CONCLUSIONS - Executive Summary:

DC® 5700 hydrolysate was evaluated for the potential to cause forward gene mutations in mouse lymphoma L5178Y ($TK^{+/-}$) cells in two nonactivated and two S9-activated assays. The nonactivated trials were conducted with nine doses (0.5, 1.0, 2.5, 5.0, 6.0, 7.0, 8.0, 10.0, and 13.0 μg/mL); the first S9-activated trial evaluated 0.5, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 μg/mL, and 0.5, 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 μg/mL were assayed in the second S9-activated trial. However, no definitive conclusions can be reached because of the inconsistency of the data and the conflicting results. In the first trial without S9 activation, a dose-related increase in total mutant colonies and the mutation frequency (MF) was observed over a dose range that included cytotoxic levels (8.0 and 10.0 μg/mL) and doses where >10% of the cells survived treatment (5.0, 6.0, and 7.0 μg/mL). MFs ranged from a 5.1-fold increase over background at 5.0 μg/mL to a 90.4-fold increase at 10 μg/mL. Using a comparable range of test material doses, the positive mutagenic response was not reproduced in the repeat nonactivated trial. In the presence of S9-activation, an ≈4-fold increase in total mutant clones and the MF was observed at 20 μg/mL in the first trial; the total growth at this dose was 29.6% of the control. Higher doses were not cloned because of excessive cytotoxicity, and lower doses were not mutagenic. In the repeat S9-activated trial, no evidence of a mutagenic response was seen. The study author provided the following explanation for the conflicting results:

> The reason for the lack of agreement is unknown. No technical problem was identified in any of the assays that could explain the differences observed. The same methodology, cells, media, and reagents were used for both trials. The only difference noted was that a 6-hour longer expression period was used in Trial II compared to that used in Trial I. The test material is a quaternary amine: a family of chemicals noted for their adverse effects on cell membranes and membrane transport. Due to the nature of the test material, the continued depression of cell growth through the 2-day expression period at toxic dose levels, and the known differences between the trials, there is a possibility that the colonies counted in the first trial could be nonmutants that survived a weakened selection process.

Although we do not necessarily agree with the study author's conclusions, we are unable to determine a plausible explanation for the lack of agreement between assay results (see Section D, Reviewers' Discussion and Interpretation of Study Results). We did, however, note that 2 months lapsed between performance of the first and second trials. Since no information was provided on test material stability, it is possible that the lack of reproducibility may be associated with test material instability under the reported storage conditions (room temperature in the dark). We conclude

that since the overall findings are equivocal, the study does not fully satisfy guideline requirements for genetic effects, Category I, Gene Mutations. The study author stated, however, that a repeat assay is in progress.

Study Classification: The study is unacceptable. Until the results of the repeat assay are available for review, no definitive conclusions can be reached regarding the mutagenic potential of DC® 5700 Hydrolysate in this test system. We recommend that information on test material stability and analytical data to support actual concentrations of the test material used in the repeat assay be included in the final report.

A. MATERIALS:

1. Test Material:

Name:	DC® 5700 Hydrolysate
Description:	White powder
Lot #:	BN029263
Purity:	81.08%
Contaminants:	See Appendix A, Test Material Composition Information
Solvent used:	Ethanol (ETOH)
Other comments:	The report indicated that the solubility of the test material in water was poor. The solubility of the test material in ETOH was found to be 25 mg/mL; therefore, ETOH was selected as the solvent of choice. Solutions of the test material were prepared fresh on the day of use. The test material was stored at room temperature protected from light.

2. Indicator Cells: The mouse lymphoma cell line, L5178Y ($TK^{+/-}$), 3.7.2C clone, was obtained from Dr. Donald Clive, Burroughs Wellcome, Research Triangle Park, NC. Stock cultures were maintained in liquid nitrogen. Cultures were periodically checked for mycoplasma contamination and exposed to aminopterin or methotrexate to maintain a low background frequency of trifluorothymidine (TFT)-resistant cells.

3. S9 Fraction: The S9 fraction was obtained from Molecular Toxicology, Inc., and was derived from the livers of adult male Sprague-Dawley rats induced with Aroclor 1254. The S9 mix contained the appropriate cofactors (3 mM NAPD and 15 mM isocitrate) and 15 to 20 µL/mL of the S9 fraction.

Prior to use, the ability of the S9 fraction to convert 3-methylcholanthrene (MCA) to a mutagenic form was determined in mouse lymphoma cells.

4. Positive Controls: Ethylmethanesulfonate (EMS) at 0.25 and 0.40 µL/mL and MCA at 2.5 and 4.0 µg/mL were used as the nonactivated and S9-activated positive controls, respectively.

5. Media/Growth Conditions: Cells were grown in RPMI medium supplemented with 10% horse serum, L-glutamine, sodium pyruvate, antibiotics, and pluronic solution. Cloning medium was growth medium without pluronic but with an increased percentage of horse serum (20%) and 0.22% agar; selection medium was cloning medium containing 3 µg/mL TFT. All cultures were maintained in a humidified incubator at 37°C in 5% carbon dioxide.

B. STUDY DESIGN:

1. Preliminary Cytotoxicity Assay: The preliminary cytotoxicity assay was performed with 10 doses of the test material ranging from 1.95 to 1000 µg/mL. Cells (density not reported) were exposed to the test material concentrations for 4 hours either in the presence or absence of S9 activation. Following exposure, cells were washed and reincubated; cell viability was determined ≈24 hours postexposure. Relative cytotoxicity was calculated and used to establish a dose range for the mutation assay that included doses yielding little or no survival to doses causing little or no cytotoxic effects.

2. Mutation Assay: Cells seeded at 6 x 10^6 cells/tube were exposed to the appropriate test material doses, solvent, or positive controls with or without S9 activation for 4 hours. Cells were washed, resuspended in growth medium, and reincubated for 2 days. Daily cell counts were determined, and cells were diluted when appropriate to maintain an optimal growth rate. At the end of the expression period, at least five doses were chosen for mutant selection.

For mutant selection, ≈1 x 10^6 cells/plate were seeded into triplicate selection medium plates. The cloning efficiency (CE) was determined by plating ≈200 cells/plate (in triplicate) in cloning medium. After 10 to 14 days of incubation, TFT-resistant colonies and the total number of viable cells were counted and the mutation frequencies (MFs) were calculated.

3. Evaluation Criteria:

a. Assay Acceptability: For the assay to be considered acceptable, the following criteria must be satisfied: (1) the CE of the solvent control should be between 60 and 130%; (2) the average suspension growth of the solvent control must show an 8-fold increase over the original cell numbers; (3) the background MF of the solvent control should range from 20 to 90 x 10^{-6}; (4) the minimum acceptable MF for the positive controls is 200 x 10^{-6}; (5) the test material must be assayed to a dose that reduces the relative growth by 10 to 20%, is at least 75% of an excessively cytotoxic dose, or is at least twice the solubility limit of the test material in culture medium; and (6) the test material MF can be evaluated only if the relative CE is ≥10% and the total number of viable clones exceeds 60.

b. Positive Response: The test material was considered positive if it induced a dose-related increase in MF that was ≥2-fold higher than the concurrent background frequency.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The preliminary cytotoxicity assay was conducted with a dose range of 1.95 to 1000 µg/mL. The report stated that doses ≥62.5 µg/mL precipitated when added to the culture medium and that the pH of the culture medium was unaffected by the test material. In the nonactivated assay, ≤1% of the cells survived treatment with doses ≥15.6 µg/mL. For the remaining nonactivated levels, percent survival ranged from 15.5% at 7.81 µg/mL to 85% at 1.95 µg/mL. Under S9-activated conditions, no cells survived exposure to doses ≥62.5 µg/mL. Percent cell survival for the remaining doses ranged from 19.6% at 31.3 µg/mL to 85.1% at 1.95 µg/mL. Based on these findings, the nine doses selected for the mutation assay ranged from 0.5 to 13.0 µg/mL -S9 and 0.5 to 60 µg/mL +S9.

2. Mutation Assay: Two nonactivated and two S9-activated mutation assays were performed with the test material; the results were as follows:

a. Nonactivated Trials: Representative results from both trials of the nonactivated assays conducted with 0.5, 1.0, 2.5, 5.0, 6.0, 7.0, 8.0, 10.0, and 13.0 µg/mL of the test material are presented in Table 1. Results from the first trial indicated that doses ≥8.0 µg/mL

TABLE 1. Representative Results from the Nonactivated Mouse Lymphoma Forward Mutation Assays with DC® 5700 Hydrolysate

Substance	Dose/mL	Relative Percent Suspension Growth[a]	Total Mutant Colonies	Total Viable Colonies	% Cloning Efficiency	Relative Percent Total Growth	Mutation Frequency[b] x 10^{-6}	Increase Over Solvent Control[c]
Solvent Control								
Ethanol	--	100.0[d]	147	764	127.3	100.0	38.9	--
	--	100.0[e]	108	679	113.2	100.0	31.8	--
Positive Control								
Ethylmethanesulfonate	0.25 µL[f]	83.1[d]	999	672	112.0	73.3	297.3	7.6
	0.25 µL[f]	90.2[e]	976	582	97.0	77.3	335.4	10.5
Test Material								
DC® 5700 Hydrolysate	2.5 µg[g]	69.6[d]	151	883	115.6	80.5	34.2	<1.0
	5.0 µg	32.4	716	725	94.9	30.7	197.5	5.1
	6.0 µg	21.3	3300	693	90.7	19.3	952.4	24.5
	7.0 µg	15.3	3004	527	69.0	10.6	1140.0	29.3
	8.0 µg	8.4	4072	776	101.6	8.5	1049.5	27.0
	10.0 µg[h]	5.1	5240	298	39.0	2.0	3516.8	90.4
	2.5 µg[g]	89.5[e]	85	714	105.1	94.1	23.8	<1.0
	5.0 µg	29.1	98	599	88.2	25.7	32.7	1.0
	6.0 µg	12.3	93	387	57.0	7.0	48.1	1.5
	7.0 µg	7.0	89	338	49.8	3.5	52.7	1.7
	8.0 µg[h]	4.2	79	178	26.2	1.1	88.8	2.8

(continued)

TABLE 1. (continued)

[a] Relative Percent Suspension Growth = $\frac{\text{Suspension Growth (test group)}}{\text{Suspension Growth (solvent control)}} \times 100$.

[b] Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Total Viable Colonies}} \times 2 \times 10^{-4}$.

[c] Fold Increase = $\frac{\text{MF of Test Dose}}{\text{MF of Solvent Control}}$.

[d] Results from trial 1.

[e] Results from trial 2.

[f] The positive control was assayed at two dose levels; results from the lowest dose were selected as representative.

[g] Results for lower doses (0.5 and 1.0 µg/mL) in both trials did not indicate a mutagenic response.

[h] Higher doses (13.0 µg/mL in trial 1 and 10.0 and 13.0 µg/mL in trial 2) were not cloned because of excessive cytotoxicity.

were severely cytotoxic (i.e., <10% survival); however, at these levels, there were marked increases in the total number of mutant colonies. Increased mutant colony counts were also seen at moderately cytotoxic doses (7.0, 6.0, and 5.0 μg/mL). In general, both mutant colony counts and the MFs exhibited a dose-dependent response, and the increased MFs, compared to the control, ranged from 5.1-fold at 5.0 μg/mL to 90.4-fold at the highest cloned dose (10.0 μg/mL). Based on these findings, the nonactivated assay was repeated using a comparable range of test material concentrations. In the second trial, excessive cytotoxicity prevented the cloning of cells exposed to the two highest test doses (10.0 and 13.0 μg/mL). Survival for the remaining doses ranged from 4.2% at 8.0 μg/mL to 119.9 μg/mL at 0.5 μg/mL. The results from the assessment of cytotoxicity in the second trial were in general agreement with the findings from the first trial; however, the results from the cloning phase of the second trial conflicted with the earlier findings. No increases in mutant colony counts were observed at any assayed dose. Although dose related increases in the MF were observed at 6, 7, and 8 μg/mL, the increases were associated with reductions in total viable colonies rather than total mutant cells.

b. S9-Activated Trials: Representative results from the two S9-activated trials are presented in Table 2. In the first trial, cells did not survive treatment with doses ≥30 μg/mL. Survival for the remaining test material levels was dose related and ranged from 34.0% at 20 μg/mL to 99.1% at 5 μg/mL; therefore, the five remaining doses (20.0, 10.0, 5.0, 1.0, and 0.5 μg/mL) were plated for expression of mutations. As shown in Table 2, both the total number of mutant cells and the MF at the 20.0-μg/mL dose level were approximately 4-fold higher that the solvent control values. At doses below 20.0 μg/mL, neither parameter was appreciably increased compared to the solvent control group. The assay was, therefore, repeated using a narrower range of test material doses (0.5, 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 μg/mL). The results of the cytotoxicity phase of the second trial were in general agreement with the earlier findings; however, no increases in mutant colonies or MFs were observed at any dose level in the second trial.

Based on the conflicting results of the first and second nonactivated and S9-activated trials conducted with DC® 5700 Hydrolysate, the study author reached the following conclusions:

TABLE 2. Representative Results from the S9-Activated Mouse Lymphoma Forward Mutation Assays with DC® 5700 Hydrolysate

Substance	Dose/mL	Relative Percent Suspension Growth[a]	Total Mutant Colonies	Total Viable Colonies	% Cloning Efficiency	Relative Percent Total Growth	Mutation Frequency[b] x 10^{-6}	Increase Over Solvent Control[c]
Solvent Control								
Ethanol	--	100.0[d]	207	786	130.9	100.0	53.7	--
	--	100.0[e]	205	674	112.4	100.0	61.3	--
Positive Control								
3-Methylcholanthrene	2.5 µg[f]	65.3[d]	707.0	619	103.2	51.6	228.4	4.3
	2.5 µg[f]	95.0[e]	656.0	501	83.5	70.6	261.9	4.3
Test Material								
DC® 5700 Hydrolysate	5.0[g]	78.2[d]	149	635	80.8	63.2	46.9	<1
	10.0	58.1	207	640	81.5	47.4	64.7	1.2
	20.0[h]	34.0	826	684	87.1	29.6	241.5	4.5
	5.0[g]	90.8[e]	197	688	102.0	92.6	57.3	<1
	10.0	71.9	163	624	92.5	66.5	52.2	<1
	15.0	47.4	195	562	83.3	39.5	69.4	1.1
	20.0	31.2	189	708	105.0	32.8	53.4	<1
	25.0	13.3	131	485	71.9	9.6	54.0	<1

(continued)

TABLE 2. (continued)

[a]Relative Percent Suspension Growth = $\frac{\text{Suspension Growth (test group)}}{\text{Suspension Growth (solvent control)}} \times 100$.

[b]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Total Viable Colonies}} \times 2 \times 10^{-4}$.

[c]Fold Increase = $\frac{\text{MF of Test Dose}}{\text{MF of Solvent Control}}$.

[d]Results from trial 1.

[e]Results from trial 2.

[f]The positive control was assyed at two dose levels; results from the lowest dose were selected as representative.

[g]Results for the lower test doses (0.5 and 1.0 µg/mL) in both trials did not indicate mutagenicity.

[h]Higher doses (30, 40, 50, and 60 µg/mL in trial 1 and 30 µg/mL in trial 2) were not cloned because of excessive cytotoxicity.

The test material, DC® 5700 Hydrolysate, was equivocal in the mouse lymphoma forward mutation assay both without and with S9 metabolic activation under the conditions of testing due to the lack of agreement between the first and second trials of the mutation assays. Repeat mutation assays will be performed to permit a more definitive evaluation.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess in general agreement with the study author that no definitive conclusions can be reached regarding the potential mutagenic activity of DC® 5700 Hydrolysate in the mouse lymphoma assay because of the conflicting results between the first and second trials of both the nonactivated and S9-activated assays. The study author stated that no technical problems were uncovered that could account for the marked differences in assay results, but speculated that the 6-hour difference in expression times between the first and second trials (41 hours in trial 1 +/-S9 and 47 hours in trial 2 +/-S9) may have enhanced the survival of nonmutant cells at selection. To support this assumption, the study author further stated that the continued depression of cell growth through the 2-day expression period in trial 1 may have permitted nonmutant cells to survive a "weakened selection process"; therefore, the colonies that arose on selection plates in the first trial probably were not true mutants (see Appendix B, Summary of Mutagenicity Testing on DC® 5700 Hydrolysate).

We disagree with the study author's conclusions for the following reasons:

1. There was no indication in the report that suspect colonies growing in the TFT-selection medium were checked for stable resistance to TFT. Without checking colonies for TFT resistance, the argument that "nonmutant" cells survived the selection process cannot be verified.

2. The statement that the additional 6-hour expression allowed more time for cells to recover from the cytotoxic effects of the test material is not supported by the data. As shown in Table 3, the results from days 1 and 2 cell counts for the nonactivated trials suggest that depression of cell growth was more severe in the second trial with a longer expression time than in the first trial.

TABLE 3. Days 1 and 2 Cell Counts in the Nonactivated Phase of the Mouse Lymphoma Forward Mutation Assays with DC® 5700 Hydrolysate

Substance	Dose/mL	Trial 1				Trial 2			
		Day 1 Cell Counts x 10^5	Percent Survival[a]	Day 2 Cell Counts x 10^5	Percent Survival[a]	Day 1 Cell Counts x 10^5	Percent Survival[a]	Day 2 Cell Counts x 10^5	Percent Survival[a]
Solvent Control									
Ethanol	--	14.6	100	14.3	100	17.4	100	18.5	100
Test Material									
DC® 5700 Hydrolysate	6.0	7.5	51.4	5.9	41.3	8.1	46.6	4.9	26.5
	7.0	6.0	41.1	5.3	37.1	5.4	31.0	4.2	22.7
	8.0	3.9[b]	26.7	5.8	40.6	4.2	24.1	3.2	17.3
	10.0	3.9[b]	26.7	3.5	24.5	2.8[b]	16.1	1.8	9.7
	13.0	1.7[b]	11.6	1.0	7.0	1.4[b]	8.0	1.0	5.4

[a]Relative Percent Survival = $\frac{\text{Cell Count (test group)}}{\text{Cell Count (solvent control)}} \times 100$; calculated by our reviewers.

[b]Day 1 cell counts with <4 x 10^5 cells were not split back.

The study author also stated that since the test material is a quaternary amine, DC® 5700 Hydrolysate may have induced adverse effects on cell membranes and membrane transport. Although we do not dispute this statement, we fail to see the relevance unless reduced permeability caused the cells not to take up the selective agent and, therefore, survive. Any inference that selective cytotoxicity toward the wild-type $TK^{+/-}$ cells, as compared to the mutant $TK^{-/-}$ cells, was related to membrane toxicity, was not borne out in the second trial. We are aware that the effect of cytotoxicity on the MF is variable and can produce a marked increase in the MF in one culture because by chance all of the wild-type cells are killed, while in a duplicate culture, the MF is low because the random cytotoxic effects were experienced by the spontaneously occurring mutants. Although single cultures were used for each test material dose in this series of assays, the difference between the doses was so small, particularly in the nonactivated assays, that the doses where a mutagenic response was observed (5, 6, 7, 8, and 10 µg/mL-S9) can, for practical purposes, be considered equivalent. Therefore, if random effects of cytotoxicity had occurred we would not have expected the uniformity of the mutagenic response in the first trial and the uniform negative response in the second trial.

We conclude that there is no single explanation for the lack of agreement between the assays. We noted, however, that information on test material stability was not included in the report and that approximately 2 months lapsed between performance of the first and second trials. It is, therefore, possible that the inability to reproduce the findings from the first trial could be related to compound instability under the reported storage conditions (room temperature protected from light). Since the results of this study are equivocal, we assess that the study is unacceptable. The study author stated that additional work is in progress; until the repeat assay is completed, no definitive conclusions can be reached regarding the potential mutagenic activity of DC® 5700 Hydrolysate in this test system.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated October 25, 1989.

F. CBI APPENDIX: Appendix A, Test Material Composition Information; Appendix B, Summary of Mutagenicity Testing on DC® 5700 Hydrolysate, CBI p. 1; Appendix C, Materials and Methods, CBI pp. 11-21.

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